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ABSTRACT

We used respondent-driven sampling among men who have sex with men (MSM) in Zagreb, Croatia in 2006 to investigate the prevalence of HIV, other sexually transmitted infections and sexual behaviours. We recruited 360 MSM. HIV infection was diagnosed in 4.5%. The seroprevalence of antibodies to viral pathogens was: herpes simplex virus type-2, 9.4%; hepatitis A, 14.2%; hepatitis C, 3.0%. Eighty percent of participants were susceptible to HBV infection (HBs antigen negative, and no antibodies to HBs and HBc antigen). Syphilis seroprevalence was 10.6%. Prevalence of Chlamydia and gonorrhoea was 9.0%, and 13.2%, respectively. Results indicate the need for interventions to diagnose, treat and prevent sexually transmitted infections among this population.

Keywords: HIV, STI, men who have sex with men, respondent driven sampling, sexual behaviours

INTRODUCTION

Croatia is a country of south-east Europe that has 4.4 million inhabitants; its capital Zagreb has 780,000 inhabitants. The surveillance system of HIV and other sexually transmitted infections (STIs) has so far relied mainly on case reporting. HIV prevalence is assessed among those who are HIV tested at clinical settings, at VCT centers and among blood donors (Begovac et al., 2006). Health care workers are required to notify all cases of HIV/AIDS and other STIs on a name basis to the Croatian National Institute of Public Health (CNIPH). The reporting of HIV/AIDS cases has been consistent over the years mainly because of a good collaboration between the CNIPH, HIV testing laboratories, voluntary counseling and testing (VCT) centers and the University Hospital for Infectious Diseases in Zagreb (UHID). UHID is the only HIV treatment center in Croatia and also serves as the reference HIV testing laboratory, where all HIV positive test results from other clinics or institutions are confirmed. The number of VCT centers in Croatia increased since the implementation of the project funded by The Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM). Before the implementation of the GFATM project which started in 2003, there were only two VCT centers, while in 2007 there were ten of them.

The first HIV case was documented in 1985 and until 2006, 608 cases of HIV/AIDS were reported. Croatia has a sexually transmitted HIV epidemic, men who have sex with men (MSM) comprise 41.8% and heterosexuals comprise 39.6% of all cases. Based on abovementioned data, it has been suggested that Croatia has a low-level HIV epidemic (Begovac et al., 2006). However, population-based seroprevalence estimates among vulnerable groups have not been available. The reporting of STIs other than HIV does not seem reliable as the reporting of HIV/AIDS as indicated by the very low number of registered cases. For example, only 24 cases of syphilis and 12 cases of gonorrhea were reported among

men in 2005 in whole Croatia. There has also been a lack of research on the prevalence of STIs, particularly among vulnerable groups such as MSM.

We conducted a survey on the prevalence of HIV, STIs and risky sexual behaviors among MSM in Zagreb, using respondent-driven sampling (RDS). We report the prevalence of HIV infection, syphilis, gonorrhea, Chlamydia, herpes virus type-2 (HSV-2) and hepatitis A (HAV), B (HBV) and C (HCV). Here we present data on HIV and STI prevalence and only the main behavioral data. Results on HIV-related risk behaviors among HIV-negative MSM from this study were described in another paper (Štulhofer et al., 2008).

The study was approved by the Ethics Committee of the UHID. Participants gave a verbal consent for participating.

METHODS

RDS has been used in various settings to recruit hard-to-reach populations and its methodology is detailed elsewhere (Heckathorn, 1997). In brief, it is a chain referral method that starts with the selection of a limited number of initial respondents (seeds) who are asked to recruit other members of the target population with coupons given by the study staff. Each recruited respondent who meets the eligibility criteria and participates in the study is given a similar number of coupons and the recruitment continues until the sample size and equilibrium are met. RDS relies on the assumption that sufficiently long referral chains obtained in at least 4-6 waves make the sample composition stable as it reaches equilibrium (Heckathorn, 1997). Equilibrium is the state whereby limited sample variation will occur no matter how many more waves are recruited. RDS sampling relies on the Markov theory of chains whereby the bias introduced by the non-random selection of initial respondents is eliminated as recruitment progresses from wave to wave. RDS can result in a probability sample using statistical weights based on the selection probability from a personal network

and recruitment patterns. One of the key advantages of RDS is that it can reach those who do not attend institutionalized settings, such as clinics, and public venues.

Study site

The study was carried out at the UHID in Zagreb. Data were collected at the hospital's HIV VCT Centre between September and December 2006. Participants provided informed consent before participating and were screened for eligibility. After completing the questionnaire, participants provided biological specimens for HIV and STI testing and received pre-test counseling. Three weeks after testing, they could receive test results and post-test counselling. All those with positive test results were referred to further care and treatment. No personal identifiers were used and all participant information was kept confidential and anonymous. Participants were given codes that were linked to the questionnaires and laboratory tests. They could collect their test results at the UHID VCT Centre by using codes.

Recruitment and sample size

For inclusion, participants were male, 18 years or older who had anal intercourse with another man in the past 12 months. Recruitment was initiated with eight initial respondents; two initial respondents were added later to enable faster recruitment. Each participant received three coupons with a two-week expiration date and was told to give these three coupons to his peers. Participants received a primary incentive of $US 18 for the participation in the survey, and $US 9 as the secondary incentive for each person they recruited. After 3.5 months of data collection, 360 participants, including initial respondents, were recruited.

Specimen collection and laboratory tests

Biological specimens were tested at the Departments for Serological Diagnostics and for Molecular Diagnostics and Flow Cytometry, UHID. Clinical specimens included blood to test

for HIV, syphilis, HSV-2, HAV, HBV and HCV. Participants self-collected urine and rectal samples, which were tested for *Chlamydia trachomatis* and *Neisseria gonorrheae*.

HIV serostatus was defined by determination of HIV p24 antigen and antibodies against HIV-1 and HIV-2 by enzyme immunoassay (EIA; Greenscreen Ultra HIV Ag-Ab, Bio-Rad, France) and reactive samples were confirmed with line immunoblot assay (LIA; Inno-LIA HIV I/II Score, Innogenetics, Belgium). Serum samples were tested for antibodies against HAV, HCV and HBV surface (HBs) and HBV core (HBc) antigens; and for HBs antigen (EIA; DiaSorin, Italy). Participants who were HBs antigen negative and had no antibodies to HBs and HBc antigens were considered susceptible to HBV infection. Immunity due to natural infection was defined if antibodies to HBs and HBc antigens were present in the absence of HBs antigen. Participants who were HBs antigen negative and had no antibodies to HBc antigen but had antibodies to HBs antigen were considered immune to HBV infection due to vaccination. Because additional tests were not performed, HBV infection in participants with only HBc antibodies was considered unclassifiable. HSV-2 seroreactivity was assessed by recombinant gG2 EIA (DiaSorin, Italy) and confirmed by immunoblot test (Focus Diagnostics, USA). Syphilis serology was assessed using the treponema pallidum hemagglutination assay (TPHA; Newmarket Laboratories Ltd, UK). All tests were performed according to manufacturers' recommendations.

Detection of *Chlamydia trachomatis* and *Neisseria gonorrheae* in urine and rectal swabs was performed by PCR (COBAS AMPLICOR CT/NG test, Roche Molecular Systems, USA).

In an attempt to shorten the diagnostic window between infection and seroconversion, we performed nucleic acid testing for HIV-1 RNA and HCV RNA in 10 sample mini-pools, which were prepared manually by mixing 100 µl aliquots of serum and plasma for detection of HCV RNA or HIV-1 RNA, respectively (Seme et al., 2007). Detection of HCV RNA was performed using COBAS AmpliPrep/COBAS AMPLICOR HCV Test, Version 2.0 (Roche

Molecular Systems, USA); and HIV-1 RNA testing used COBAS AmpliPrep/COBAS AMPLICOR HIV-1 Monitor Test, Version 1.5, UltraSensitive specimen preparation (Roche Molecular Systems, USA). If HCV and HIV-1 RNA positive pools were found, individual testing of samples was performed.

Questionnaire and coupon administration

The questionnaire was self-administered and included items on socio-demographic characteristics, knowledge about HIV and STIs, drug and alcohol use, sexual behaviors, STI diagnoses and symptoms, and access to and use of HIV prevention services. Data were also collected on participants' social network sizes (assessed as the number of MSM they know by name, are 18 years or older, who live in Zagreb and have seen in the past three months) and reasons for participation. At the end of the data collection, participants were given three coupons that had the basic information about the interview site and the study, and were given explanations on the coupon distribution. Information was collected on the number of people who refused to accept coupons and reasons for refusal once participants came back to the survey site to collect test results and secondary incentives. During RDS, such brief questionnaire is used to assess how many people refused to accept coupons and serves to estimate the refusal to participate in the study.

Analyses

RDS Analysis Tool (RDSAT) version 5.6 was used to analyze data. RDSAT calculates the population estimates and confidence intervals around these estimates by using recruitment linkages and the self reported social network sizes. All indicators calculated by RDSAT are presented as population proportion estimates ("RDS estimates"), which account for differential social network sizes and homophily. Results are presented as point estimates with 95% confidence intervals (CI).

RESULTS

The main results on prevalence of infections and sexual behaviors are shown in Table 1.

Patterns of recruitment

Recruitment per initial respondent varied - the oldest initial respondents were the least successful recruiters, which could indicate small social network sizes or the unwillingness of their peers to participate. The number of waves per initial respondent ranged from 0-13, and the highest recruitment was achieved in the fourth wave. The reported social network sizes ranged from 1-600 (median, 13; interquartile range [IQR], 6-21). A total of 1062 coupons were given to study participants. Information on the number of coupons given to peers and number of people who refused to accept coupons is available from 284 participants who came back to the site to collect the test results. Overall, 424 persons refused to accept a coupon, and 515 accepted them, which gives the coupon refusal rate of 45.1%. The most common reasons for refusing to accept coupons were: lack of interest (35.1%); lack of time (25.7%); fear of disclosing being an MSM (20.3%); certain that he cannot get infected with HIV (3.8%); tested elsewhere (3.1%); does not want to know HIV status (2.4%); the survey site is not adequate (1.7%); concerns that the survey is not anonymous (1.7%). Those that were offered coupons were most often described as close friends (52.8%), acquaintances (28.7%), someone who was recently met (6.1%), sexual partners (2.4%), while 10% did not provide any information. Most of participants said that they were recruited by friends (58.1%), acquaintances (21.6%) and sexual partners (11.1%), while 7.9% said that they saw for the first time a person who gave them coupons and 1.2% reported relatives as recruiters. Six participants who came to the study site were found ineligible, and no one withdrew from the survey once they provided an informed consent. Those ineligible did not have an anal intercourse with another man in the past 12 months.

Socio-demographic characteristics

The age of participants ranged from 18-69 years (median, 27; IQR, 23-32). Though most participants self-identified as homosexual, bisexual orientation was reported by 34.9%. One participant declared himself as a heterosexual. Among others, one said he was asexual and two were indecisive. Thirty-six percent of respondents had a university degree, and 57.3% had completed a secondary school.

Viral sexually transmitted infections

HIV infection was found in 18 subjects or 4.5% (95% CI 2.2-7.3) (Table 1). HIV RNA was detected in 14 respondents. Antibodies to HSV-2 were detected in 9.4% of participants. The prevalence of HCV antibodies was 3%, whereas the carriage of HBs antigen was found in only two participants. The vast majority (80%) of participants were susceptible to HBV infection (HBs antigen negative, and no antibodies to HBs and HBc antigen). Evidence of immunity due to natural HBV infection was found in 22 (7.7%) participants. Only 11.2% were considered immune due to vaccination (HBs antigen negative, anti-HBs positive, anti-HBc negative). The status of HBV infection could not be determined in five participants with only HBc antibodies. Two of nine participants with HCV antibodies had detectable HCV RNA. As none of our participants had a negative antibody test and a positive HCV and HIV RNA PCR test, no case of acute HIV or HCV infection was diagnosed. Only one person with HCV antibodies said he had every injected drugs.

Slightly more than a half of respondents never received an HIV test (51.9%). Eight respondents self-reported as HIV positive, and they were confirmed as positives. Seventy-eight percent of participants returned to the study site to collect the results.

Bacterial sexually transmitted infections

Syphilis seropositivity was found in 10.6% of participants. *N. gonorrheae* and *C. trachomatis* were detected in 13.1% and 7.8% of rectal samples, respectively. Chlamydial infection from urine was found in 2.0% of samples, and gonococcal in only 0.4%. Altogether, 9% of MSM

had Chlamydial infection and 13.2% had a gonococcal infection. Two men out of 76 diagnosed with Chlamydia and gonorrhea reported current symptoms of urethral and/or anal discharge.

Sexual behaviors and reason for participation

The main results on sexual behaviors are shown in Table 1. The median age of first sexual encounter with another man was 19 years (IQR, 7-21). The median number of partners ever was 6 (IQR, 3-12), and in the past five years it was 5 (IQR, 2-10).

Analysis of those with casual partners in the past 12 months revealed that at least one unprotected intercourse with such partners was almost twice frequent during oral (88.9%) than anal sex (45.5%) (Table 1). Approximately half of MSM had steady partners at the time of their interview, and 75% of the current or last steady partnerships were estimated as mutually faithful. During the past 12 months, unprotected oral sex with foreigners was more common (21.2%, 97/360) than unprotected anal sex (6.7%, 28/360). Most of those who had partners from countries outside Croatia said that they came from western Europe. Thirteen percent of MSM reported group sex in the past 12 months and slightly more than a half of these used condoms last time they had group sex. Commercial sex was relatively rare and, altogether, 6% (28/360) of MSM either bought or sold sex ever. Slightly more than a half of participants had female sexual partners, though currently only 6.9%. Ever injecting drugs was reported by 5.3% (15/360); three of them said that they shared needles at least once. Sixty-four percent of participants consider themselves as being at small or moderate risk of HIV.

The main reason given for participation in the study was STI (90.2%, 321/360) and HIV (68.6%, 236/360) testing.

DISCUSSION

We found an HIV prevalence of 4.5% in predominantly young MSM from Zagreb who had anal intercourse in the previous 12 months. UNAIDS and WHO have defined a concentrated

epidemic when HIV prevalence is consistently above 5% in a given population (UNAIDS and WHO, 2003). Thus, it seems that Croatia might be approaching a concentrated epidemic among MSM. There are little data on HIV seroprevalence in southeastern Europe or from other countries neighboring Croatia. Bino et al. conducted an RDS study on MSM in Tirana, Albania and found a seroprevalence of 3.0% in 2005 (Bino et al., 2006). Unlinked anonymous surveys in Slovenia reported seroprevalences of 0.0 to 3.4% from 1993 to 2002 (Klavs and Poljak, 2003). The HIV prevalence in our study was lower than the one found in surveys in urban centers of Great Britain (8.6%-13.7% in London, Brighton and Manchester) and five large cities in the US (range by city: 18%-40%) (Centers for Disease Control and Prevention, 2005).

HSV-2 prevalence found in our study (9.4%) was lower than the 19% to 66% range reported in homosexuals from various settings worldwide (Smith and Robinson, 2002). Younger age is associated with a lower HSV-2 seroprevalence particularly in at risk populations (Smith and Robinson, 2002), however, this only partially explains our findings.

Based on the absence of hepatitis A antibody (85.8%) and anti-HBs antibody and anti-HBc antibody (80%), a large proportion of our MSM are susceptible to hepatitis A and B infection. As MSM are at risk of acquiring hepatitis A and B, there is a need for immunization against those viruses. Unfortunately, there seems to be little awareness for the need for vaccination of MSM among health care professionals. The cost of hepatitis A and B vaccination in currently not covered by the Croatian National Health Insurance system and depends on individual payment. The prevalence of hepatitis C infection in presumably non-injecting MSM in our study was 2.8% (8/344, 95% CI 0.8-4.6), which is relatively high compared to studies from the United Kingdom (0.9%) and USA (1.5%) (Balogun et al., 2003; Buffington et al., 2007). This might support the view that HCV can be sexually transmitted

or it could indicate underreporting of IDU by MSM. We found no HIV infection among those with antibodies to HCV.

Syphilis prevalence (10.6%) was similar to that found among young MSM in St Petersburg, Russia (10.4%) (Amirkhanian et al., 2006). However, the prevalence in our study indicates the lifetime exposure to *Treponema pallidum* infection as no tests in this study were done to estimate the proportion of new cases of syphilis. Rectal Chlamydial and gonococcal infections were more frequent than urethral infections and were predominantly asymptomatic. There is scarcity of population-based studies on STIs among MSM articularly in eastern Europe but the prevalence rates in this study appear to be slightly higher than the rates found among men attending STI clinics in London and in San Francisco. Among MSM attending a genitourinary medicine clinic in London, the prevalence of rectal and urethral Chlamydial infection was 6.5% and 4.3%, respectively, and rectal and urethral gonococcal infection 7.3% and 7.2%, respectively (Benn et al., 2007). In clinical settings in San Francisco the numbers were 7.9% and 5.2% for rectal and urethral Chlamydia, respectively, and 6.9% and 6.0% respectively, for rectal and urethral gonorrhoea (Kent et al., 2005).

Though current partnerships with women were not common, of notice is that slightly more than a third of respondents self-identify as bisexual. We cannot assess from our study whether these men are forth coming to their female partners about their sexual behavior and to what extent failure to disclose sexual activities may be due to stigmatization of MSM behavior. Further research should characterize the sexual networks of bisexual men and assess their potential impact on population transmission of STIs.

As indicated by respondents, the main reason for participation in the study was STI testing. This might imply that existing STI services are not adequate for MSM or do not meet their needs. This lack of MSM-specific STI screening services in Croatia might contribute to STIs

being missed, particularly in the light of the high proportions of asymptomatic rectal infections.

In that sense, it is encouraging to see that most MSM returned to the study site to collect test results and receive post-test counseling.

There are several important findings from this study. Firstly, as slightly more than a half of the study population (52%) never had an HIV test, there is a clear need to increase HIV testing in MSM. Secondly, given the potential increased susceptibility to HIV infection among MSM with STIs, screening and treatment of STIs should be important HIV prevention strategies. Thirdly, the results suggest that there are behavioral factors that might increase the spread of HIV and STIs in the MSM population, which indicates the need to scale-up behavioral programs aiming at increasing condom use during anal and oral sex and decreasing the number of partners.

Due to the biases that are inherent in sampling hard to reach populations and RDS, the generalizations of the findings to MSM living in Zagreb has certain limitations. Non-response bias is one of the main disadvantages of RDS. It is assessed indirectly, by asking those who came to the survey site to collect secondary incentives how many of those approached refused to accept coupons and what the reasons for that might have been. We do not know whether those who refused were at lower or at higher risk for HIV. A third of those who refused a coupon said they were not interested in participating. This indicates the need to advocate for the usefulness of such research to the MSM community. A minority of respondents were recruited by someone they just met. We think that this is not the major source of bias because the assumption in RDS is not that each respondent has to recruit randomly, but rather the aggregate recruitment should reflect the composition of their social networks. The study results are also not generalizable to those MSM that did not have anal intercourse in the past 12 months. The socio-demographic structure of the sample suggests

that it represents less well MSM older than 35 and those with lower educational status as one in five men in the sample was older than 35 and every third had a university degree. There is anecdotal evidence that older MSM might be married because of social pressure, or reluctant to attend the study site which is located in the public hospital. However, such assumptions require exploration in qualitative studies. Nucleic acid amplification tests (NAAT) are not cleared by the US Food and Drug Administration for the detection of gonorrhoea and Chlamydia in rectal specimens. Renault et al., reviewed the data on the sensitivity and specificity of different NAATs and reported a mean sensitivity of 53.6% for rectal gonorrhoea and 91.2% for rectal Chlamydia by the PCR method we used (Renault et al, 2006). The mean specificity for the PCR method was 99.1% for rectal gonorrhoea and 95.8% for rectal Chlamydia infection (Renault et al., 2006). Since we used only one screening method for detection of gonorrhoea and Chlamydia our results should be interpreted with caution. A lower degree of sensitivity of the PCR might imply that we underestimated the prevalence of gonorrhoea and to a lesser extent that of Chlamydia.

In predominantly young MSM from Zagreb who had anal intercourse in the previous 12 months, the prevalence of HIV infection (4.5%) is approaching the 5% threshold that defines a concentrated epidemic. Behavioral data and data on syphilis and rectal gonorrhea indicate the presence of high-risk sexual practices. There is a need to increase HIV testing uptake among MSM in Croatia, develop STI screening services, and implement more comprehensive behavioral change programs. Existing surveillance data on syphilis and gonorrhea underestimate the number of infections and population-based surveys such as this one may have better public health utility and should be conducted at regular intervals.

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Table 1. Prevalence of HIV and other sexually transmitted infections and sexual behaviors among 360 men who have sex with men in Zagreb, Croatia, 2006

Biological data	RDS population estimates % (95% CI)	n/N
HIV-1/2	4.5 (2.2-7.3)	
Herpes simplex virus type 2	9.4 (4.8-14.4)	22/360
Hepatitis A	14.2 (9.1-19.8)	44/360
Hepatitis C	3.0 (1.1-5.3)	9/360
Antibodies to hepatitis B core antigen[a]	9.2 (5.2-13.1)	28/360
Antibodies to hepatitis B surface antigen[a]	17.9 (13.6-22.8)	76/360
Hepatitis B surface antigen	0.9 (0.0-1.2)	2/360
Syphilis[b]	10.6 (6.6-15.1)	31/360
Chlamydia trachomatis (urine and/or rectal)[c]	9.0 (6.2-12.3)	46/360
Neisseria gonorrheae (urine and/or rectal)[c]	13.2 (9.1-18.5)	42/359
Sexual behaviors with men		
Unprotected anal intercourse with at least one casual partner in the past 12 months[d]		
Yes	45.5 (39.6-57.2)	113/256
No	54.5 (42.8-60.4)	143/256
Used condom during the last anal intercourse with the current steady partner[e]		
Yes	37.3 (26.1-54.1)	66/171
No	62.7 (45.9-74.0)	105/171
Unprotected oral sex with at least one casual partner in the past 12 months[f]		
Yes	88.9 (82.7-92.9)	259/297
No	11.1 (7.1-17.3)	38/297
Had group sex in the past 12 months		
Yes	13.3 (10.1-17.3)	80/ 360
No	86.7 (82.7-89.9)	280/360
HIV status of current or last steady partner		
Unknown	47.5 (40.9-53.4)	130/336
Known	52.5 (46.6-59.5)	206/336
Sexual behaviours with women		
Ever had oral, vaginal or anal sex with women		
Yes	52.7 (46.3-59.7)	193/ 357
No	47.3 (40.3-53.7)	164/357
Currently in the relationship with a woman or married		
Yes	6.9 (2.5-11.7)	16/ 355
No	93.1 (88.3-97.5)	339/355
Used condom during the last intercourse with women		
Yes	68.2 (50.0-75.8)	98/175
No	31.8 (24.3-50.2)	77/ 175

RDS, respondent-driven sampling. Totals vary because of missing data or subgroup analysis.
[a] the test results of 11.2% participants indicated immunity due to HBV vaccination and 7.7% participants showed evidence of immunity due to natural HBV infection. Overall, 80% of participants had no immunity to HBV [b] *Treponema pallidum* haemagglutination test; [c] Nucleic acid amplification testing; [d] 101 respondents said that they did not have anal intercourse with a casual partner in the past 12 months; three did not answer the question; [e] 174 respondents reported current steady partners; three did not answer the question; [f] 61 respondents said that they did not have oral sex with a casual partner in the past 12 months and two did not answer the question.